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Progression of overt nephropathy in non-insulin-dependent diabetes

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Progression of overt nephropathy in non-insulin-dependent diabetes. The detection of overt albuminuria (> 300 mg/g creatinine) in the absence of azotemia was used to diagnose early nephropathy in 34 Pima Indians with NIDDM of 16 ± 1 years duration. Differential solute clearances were performed serially to define the course of the glomerular injury over 48 months. At baseline, the GFR (107 ± 5 ml/min), filtration fraction and sieving coefficients of relatively permeant dextrans (< 52 Å) were all depressed below corresponding values in 20 normoalbuminuric Pima Indians with a similar duration of NIDDM. Over the ensuing 48 months the GFR (−34%) and filtration fraction (−13%) in the nephropathic patients declined further. The sieving coefficients of large, nearly impermeant dextrans (> 56 Å radius) increased selectively and fractional clearances of albumin and IgG increased correspondingly by > 10-fold. Analysis of the findings with pore theory revealed: (1) a progressive decline in pore density and the ultrafiltration coefficient (K_f); and (2) broadening of glomerular pore-size distribution that resulted in greater prominence of large pores (> 70 Å radius). We conclude that increasing loss of intrinsic ultrafiltration capacity is the predominant cause of the early and progressive decline in GFR that follows the development of nephropathy in NIDDM. We speculate that progressive impairment of barrier size-selectivity contributes to but does not fully account for the increasingly heavy proteinuria that is observed early in the course of this disorder.

Between 30 and 45% of patients with insulin-dependent diabetes mellitus (IDDM) ultimately develop diabetic nephropathy. This distinctive renal injury is ushered in by a decade or more of glomerular hyperfiltration followed by a progressive increase in the rate of urinary albumin excretion. Initially, the albuminuria spans a modest range (30 to 300 mg/24 hr) quantifiable only with sensitive immunochemical techniques, and referred to as "microalbuminuria". Because microalbuminuria has been associated with a rapid rate of glomerular filtration (GFR) and little or no alteration in glomerular morphology, it has been referred to as a stage of incipient nephropathy [1]. Only when albuminuria increases into a range measurable by dipstick (> 300 mg/24 hr) and referred to as "macroalbuminuria", has a progressive decline in the GFR and unequivocal histopathological evidence of diffuse and/or nodular glomerulosclerosis been observed. By convention therefore, the development of macroalbuminuria has been used to define the onset of overt diabetic nephropathy [1]. The latter appears to advance irrevocably in most affected patients and leads eventually to end-stage renal failure.

The natural history of nephropathy in patients with non-insulin-dependent diabetes mellitus (NIDDM) has been more difficult to characterize than in IDDM. This is particularly true of Caucasians in whom the onset of NIDDM is difficult to pinpoint and occurs at an advanced age [2, 3]. The confounding factors include an effect of aging *per se* to lower the GFR [4], a high frequency of co-existent renal disease unrelated to diabetes beyond the age of 50 years [5–7], and a high mortality rate from cardiovascular disease [8–10]. The latter phenomenon limits the full expression of the natural history of diabetic nephropathy, with the result that only 3 to 8% of such patients have been observed to progress eventually to end-stage renal failure [10].

To circumvent the confounding influence of advanced age on glomerular function in NIDDM, we conducted a study of the Pima Indians of the Gila River Indian Community of Arizona. They have an extraordinarily high incidence rate of NIDDM, which peaks between the third and fifth decades [11]. Judged by initial hyperfiltration [12], a high prevalence of micro- and macroalbuminuria [13, 14], histopathological evidence of intercapillary glomerulosclerosis [15], and a high incidence of progressive renal failure [16], the nephropathy associated with NIDDM in this population appears to be similar to that observed in IDDM. The purpose of the present study was to evaluate the course of overt diabetic nephropathy in this population. To do this we identified non-azotemic subjects with macroalbuminuria and performed serial studies of glomerular function over a 48 month interval.

Methods

Subject selection

The Gila River Indian Community and the nearby community of Ak-chin are inhabited primarily by Pima and the closely related, Tohono O'odham Indians. Those between the ages of 18 and 60 years, whose heritage was at least 50% Pima, Tohono O'odham, or a mixture of these two tribes, and who had NIDDM of ≥ 5 years duration were potential candidates for this study. One hundred eighty-six of these individuals submitted urine specimens on three occasions, each separated by at least seven days. The urine was assayed for albumin and expressed as an

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Table 1. Clinical features at the baseline examination

	Normoalbuminuria	Macroalbuminuria
Men, Women	8, 12	18, 16
Age *years*	44 ± 2	47 ± 1
Duration of diabetes *years*	13 ± 1	16 ± 1[a]
Weight *kg*	93 ± 6	89 ± 3
BMI kg/m^2	34.2 ± 1.9	31.7 ± 1.1
Systolic BP *mm Hg*	121 ± 4	134 ± 3[a]
Diastolic BP *mm Hg*	74 ± 2	86 ± 2[a]
Fasting glucose *mg/dl*	228 ± 16	240 ± 12
Hemoglobin A_1 %	11.4 ± 0.6	12.2 ± 0.3
Serum creatinine *mg/dl*	0.69 ± 0.02	0.89 ± 0.04[a]

[a] $P < 0.05$ vs. normoalbuminuria

albumin-to-creatinine concentration ratio (A/Cr ratio). Thirty-four subjects with a ratio above 300 mg/g in at least two of the three urine specimens were judged to have macroalbuminuria, and hence overt diabetic nephropathy. Each agreed to participate in a study of glomerular function. Twenty individuals with long-standing NIDDM and a normal A/Cr ratio (< 30 mg/g) on all three screening examinations volunteered to serve as controls.

Some clinical features of each group are summarized in Table 1. The nephropathic group had a higher proportion of males than the normoalbuminric controls. Age did not differ between the two groups, but the duration of NIDDM was slightly shorter in the control than the nephropathic group, 13 ± 1 versus 16 ± 1 years, respectively. Judged by body mass index, the degree of obesity was similar, as was the level of chronic glycemia (Hemoglobin A_1) upon entry into the study (Table 1). Two control subjects and 15 nephropathic subjects were receiving insulin therapy at the time of study. Approximately one-half of the members of each group were taking an oral hypoglycemic agent. Nephropathic subjects differed from controls in that arterial blood pressure and the serum creatinine level were higher, albeit still within the normal range (Table 1).

Study protocol

Cross-sectional studies. Each subject consented to be studied according to a protocol that had been approved by the review boards of all participating institutions, namely Stanford University, California, The Cleveland Clinic, Ohio, the National Institute of Diabetes and Digestive and Kidney Diseases, Maryland, and the Gila River Indian Community Tribal Council, Arizona.

Subjects were admitted to either the Clinical Research Center in the Phoenix Indian Medical Center or to an especially equipped room of the Hu Hu Kam Memorial Hospital in Sacaton, Arizona for the performance of differential solute clearances. Blood pressure was determined in the sitting position and mean arterial pressure (MAP) calculated as the diastolic pressure plus one-third of the pulse pressure. An indwelling plastic cannula was then inserted into the antecubital vein of each arm, one for collecting blood samples and the other for infusing clearance markers. Serum albumin, immunoglobulin G (IgG) and creatinine concentrations were assayed, and the oncotic pressure of plasma was determined. After the bladder was emptied by spontaneous voiding, urine was examined to determine the A/Cr and IgG-to-creatinine (IgG/Cr) ratios. A diuresis was next initiated with an oral water load of either 10 ml/kg or 1500 ml in the case of subjects weighing > 150 kg. Clearance markers were infused beginning with a loading dose of 30% iothalamate (300 mg plus 3 mg/kg for each kg above 100 kg), 20% para-aminohippuric acid (PAH, 16 mg/kg) and 10% dextran 40 (150 mg/kg). Each clearance marker was then delivered by an infusion pump to maintain iothalamate and PAH plasma concentrations constant at 1.5 and 2.0 mg/dl, respectively [12]. Dextran 40 was infused at half the rate of iothalamate. After a 60-minute equilibration period, the bladder was again emptied by voiding, and four carefully timed urine collections were made at approximately 20 minute intervals. Blood was drawn to bracket each urine collection. To prevent the formation of PAH-glucose adducts in urine, 4 ml of each urine collection was promptly alkalinized by addition of 30 μl of 4 M NaOH [17, 18].

The average urinary clearance of iothalamate was equated with the GFR. In light of earlier demonstrations in non-azotemic diabetic subjects of normal renal PAH extraction, the corresponding clearance of PAH was used as a measure of renocortical plasma flow rate (RPF) [19, 20]. Fractional clearances of dextran macromolecules in the 32 to 60 Å radius interval were calculated for the second or third timed collection period by dividing the clearance of each dextran by that of iothalamate. Fractional clearances of the endogenous proteins, albumin and IgG were determined simultaneously.

Longitudinal studies. The nephropathic group underwent longitudinal study for a period of 48 months. Serial clearances of iothalamate, PAH and endogenous proteins were repeated at six month intervals. Dextran sieving measurements were performed at baseline and after 48 months, with one exception. The exception was a subject who advanced to end-stage renal failure after 37 months. In this case the exit study of dextran sieving was performed after 32 months of follow-up. Four patients were lost to follow-up; the remaining 30 members of the nephropathic cohort completed the 48 month study. The baseline renal function of the four subjects who dropped out did not differ significantly from those who completed follow-up. The primary physicians of the participating nephropathic patients prescribed angiotensin converting enzyme inhibitors (ACEi) for 13 subjects during the course of the study. Of these eight reported taking the ACEi at only one examination, three at two examinations and two at four examinations. Consequently, six subjects (20%) were on ACEi at some time during the first year, one (3%) during the second year, three (10%) during the third year, and eight (27%) during the fourth and last year of the study. The relatively widespread use of ACEi during the last study year coincided with the publication of two reports which indicated a renoprotective effect of this agent in patients with diabetic nephropathy [21, 22].

Laboratory procedures

The concentrations of albumin and IgG in serum of all subjects and in the urine of nephropathic subjects were determined by immunoprecipitation using a semi-automated nephelometer (Behring Diagnostics, Somerville, NJ, USA). The concentrations of albumin and IgG in the urine of control subjects were at or below the lowest standard of the nephelometric assay, and were determined instead with a sensitive enzyme-linked immunosorbent assay [23]. All samples were stored at −70°C until the day of assay, which was performed within 30 days of the collection of the sample.

A high pressure liquid chromatography system with a sensitive ultraviolet light detector was used to assay iothalamate and PAH

at 236 nm (Instrumentation Shimadzu #6A, Kyoto, Japan). Ultrafiltrates of plasma and diluted urine were injected onto a reverse phase column (#C18, 5 μ Ultrasphere, Beckman, San Ramon, CA, USA). The mobile phase was 3.5% acetonitrile in 10 mM triethylamine at a pH of 3.5, and the flow rate was 1.0 ml/min. Iothalamate and PAH concentrations were determined from the peak area of each solute, corresponding to column retention times of 14 and 10 minutes, respectively [12].

Separation of dextran 40 in urine and protein-free filtrates of plasma into narrow fractions was achieved by high pressure liquid chromatography using two columns in series (Ultrahydrogel 250 and 500; Waters Division, Millipore Corp., Milford, MA, USA). The columns were calibrated with four narrowly-dispersed dextran fractions of known molecular weight (9.9, 25.6, 53.5 and 72.6 kD, respectively), provided by Dr. K. Granath of Pharmacia Fine Chemicals (Uppsala, Sweden). Dextran concentration was measured using a refractive index detector (Instrumentation Shimadzu #RID-6A). An integrator (Spectraphysics #4270, San Jose, CA, USA) was used to divide the chromatogram into four slices per minute during the 40 minute run. The integrated area of each slice was equated with the dextran concentration at the corresponding retention time. Molecular weight (MW) was calculated from its relationship with retention time and molecular radius (r_s) was then computed from molecular weight using the equation:

$$r_s = 0.33 \times (MW)^{0.463}$$

The interassay coefficient of variation for fractional dextran clearance at each size interval varied between 3.3 and 10.5%.

Oncotic pressure of plasma was determined by membrane osmometry using a Wescor 4400 colloid osmometer (Wescor Inc., Logan, UT, USA), and the concentration of creatinine by a rate-dependent modification of the Jaffe reaction [24].

Analysis of glomerular membrane-pore structure

To characterize the size-selective properties of the glomerular barrier, we employed a theoretical model which represents the glomerular capillary wall as a heteroporous membrane that is perforated by cylindrical pores with radii which are assumed to follow a lognormal distribution [25]. The two parameters which characterize this distribution are the mean pore radius (u) and the standard deviation about the mean (S) of the lognormal pore size distribution. This theoretical model also estimates volume flows and fluxes and the dextran concentration along the length of the glomerular capillaries, thereby permitting computation of an ultrafiltration coefficient (K_f), which is the product of effective hydraulic permeability and total glomerular capillary surface area in the two human kidneys. An additional membrane parameter that can be derived from K_f and pore radius is the ratio of effective pore area-to-length (S′/l), which is closely related to the apparent number of glomerular pores in the two kidneys. Provided that pore length (l) does not differ between the two groups, changes in S′/l would be predicted to be nearly proportional to changes in the total number of pores in the membrane (pore density) [26].

To evaluate the influence of GFR determinants on convective and diffusive transmembrane transport, the model requires knowledge of GFR, RPF, π_A and the transcapillary hydraulic pressure difference, ΔP [25, 26]. The latter quantity cannot be directly measured in humans, but has been estimated by two different indirect methods to range from 35 to 40 mm Hg in healthy subjects [27, 28]. Micropuncture determinations in diabetic rats have revealed moderate hyperglycemia to be associated with a selective or disproportionate fall in preglomerular (afferent) segmental vascular resistance. This permits a greater fraction of arterial pressure to be transmitted into glomerular capillaries, thereby increasing ΔP [29, 30]. Based on these findings, we infer that ΔP is unlikely to have been below the normal range in the diabetic subjects of the present study, and arbitrarily assigned a value of ΔP = 40 mm Hg to each subject for the purpose of calculating membrane parameters.

Statistical analysis

Results are expressed as the mean ± SE, except for variables with highly skewed distributions, such as the urinary protein excretion ratios and fractional clearances, which are expressed as medians and ranges. All P values are for two-sided tests, and results are deemed significant if $P < 0.05$. Age and sex were included as covariates in these analyses. Urinary A/Cr and IgG/Cr ratios and the corresponding fractional clearances of each protein were analyzed after a logarithmic transformation. Paired tests (t-tests or signed rank) were used to evaluate differences from baseline at the 48 month examination in nephropathic patients.

Differences between groups among fractional clearances of discrete dextrans at 2 Å radius intervals were first examined by t-tests with no adjustment for multiple comparisons. Differences among the entire fractional dextran clearance profiles were then analyzed by deriving membrane parameters from the theoretical model. The membrane parameters of the nephropathic group were then compared to those of controls using a nonparametric test (Kruskal-Wallis). A paired analysis was used to compare membrane parameters after 48 months in the nephropathic group to corresponding values at baseline.

Not all nephropathic patients attended the scheduled six monthly studies regularly. To assess serial changes in the quantities of interest, we used two statistical methods which take missing values into account. Linear regression analysis was used to derive a slope from the serial determinations of GFR and the fractional clearances of albumin and IgG [31]. We also used a mixed-effect model which combines spline fitting for each serially measured parameter with a random effect of the follow-up time [31, 32].

Results

Cross sectional observations

Renal protein handling and filtration dynamics upon entry into the study are summarized in Table 2. Because of the higher proportion of males in the nephropathic than the control group, the glomerular flows (GFR and RPF) are expressed per 1.73 m^2 of body surface area, so as to take gender differences in body size into account. The mean GFR in the control group was higher by 23 ml/min/1.73 m^2 than the corresponding value that we have reported previously [12] for non-diabetic Pima Indians (110 ± 4). This finding suggests that long-standing NIDDM in the absence of detectable nephropathy is associated with hyperfiltration [12]. Mean RPF in the control group was also elevated, in this case, by 112 ml/min/1.73 m^2 above the corresponding value (560 ± 25) observed in non-diabetic Pima Indians [12]. In nephropathic patients as well, the RPF was elevated, but the GFR had "fallen back" into the normal non-diabetic range, and was significantly

Table 2. Glomerular function at the baseline examination

	Normoalbuminuria	Macroalbuminuria
Fractional clearance of albumin $\times 10^{-3}$[a]	0.001 (0.0001–0.004)	0.148 (0.030–6.73)[b]
Fractional clearance of IgG $\times 10^{-3}$[a]	0.002 (0.0002–0.006)	0.050 (0.012–2.68)[b]
GFR *ml/min/1.73 m²*	133 ± 7	107 ± 5[b]
RPF *ml/min/1.73 m²*	672 ± 34	658 ± 28
Filtration fraction	0.20 ± 0.009	0.17 ± 0.006[b]
π_A *mm Hg*	21.6 ± 0.5	21.0 ± 0.4
MAP *mm Hg*	90 ± 3	102 ± 2[b]
Estimated K_f *ml/min · mm Hg*[a]	9.8 (7.5–11.6)	6.6 (6.0–8.8)[b]

[a] Median (range)
[b] $P < 0.05$ vs. normoalbuminuria

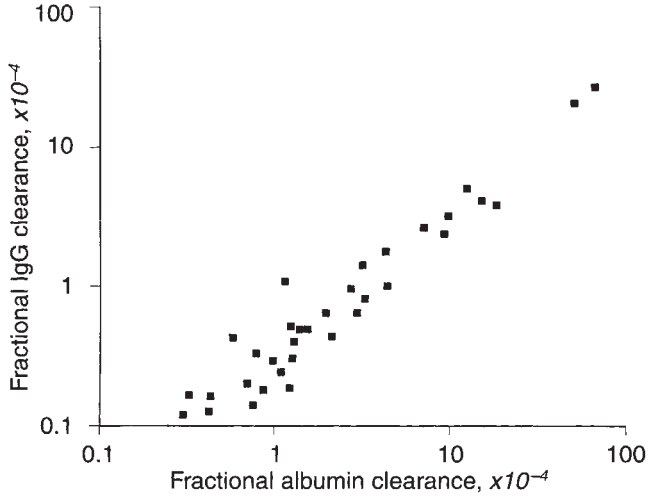

Fig. 1. *Relationship between fractional clearances of IgG and albumin in macroalbuminuric subjects at baseline (r = 0.99, P < 0.001).*

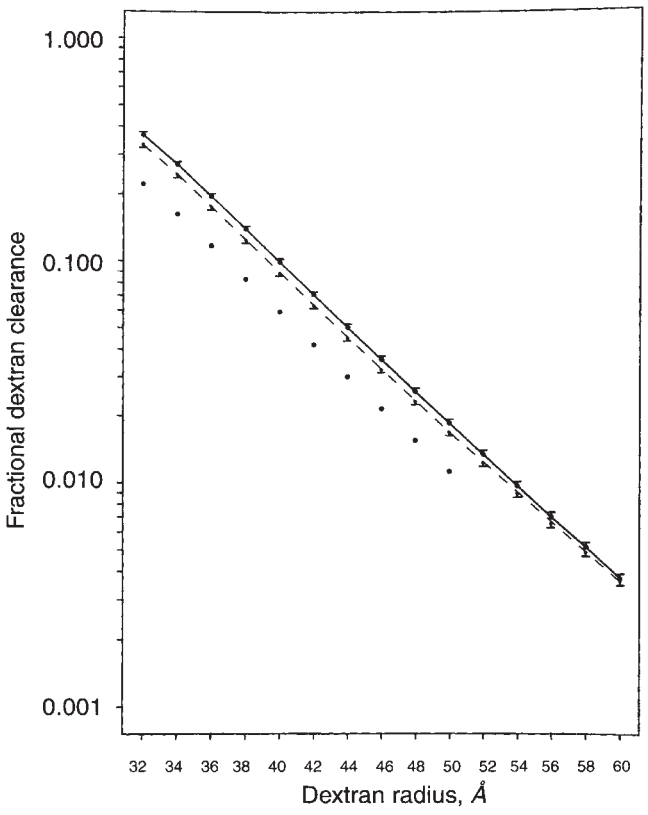

Fig. 2. *Dextran sieving curves in macroalbuminuric subjects at baseline (dashed) vs. normoalbuminuric controls (solid) (*P < 0.05 vs. control).*

lower than the corresponding value in the non-nephropathic control subjects: 107 ± 5 versus 133 ± 7 ml/min/1.73 m², respectively. The selective reduction in GFR in the nephropathic group resulted in a depressed filtration fraction, averaging 0.17 versus 0.20 in the control group (Table 2). Whereas π_A was similar in the two groups, the nephropathic group exhibited significant arterial hypertension (Table 2). Thus, macroalbuminuria in non-azotemic diabetic Pima Indians was accompanied by a fall in GFR into the normal range, a depressed filtration fraction, and the development of hypertension.

The A/Cr ratio and fractional albumin clearance in the nephropathic group were elevated above control values by two to three orders of magnitude (Table 2). The corresponding IgG/Cr ratio and fractional IgG clearance were also markedly elevated, albeit slightly less than in proportion to corresponding values for albumin (Table 2). Of note, the fractional clearances of IgG and albumin in the nephropathic group were strongly related (r = 0.99), suggesting that the glomerular permeability to each protein increased in parallel (Fig. 1).

The mean dextran sieving profiles for each group are illustrated in Figure 2. In the nephropathic subjects, dextran sieving coefficients at the low radius end of the sieving profile (32 to 50 Å interval) were significantly depressed below corresponding values in the control subjects. The sieving coefficients for large, nearly impermeant dextrans (radii 52 to 60 Å) did not differ between the two groups (Fig. 2).

The computed pore-size distribution for each group is illustrated in Figure 3. The median pore radius μ is smaller (43.8 vs. 46.1 Å, $P < 0.01$) and the standard deviation of the distribution (S) is larger (1.204 vs. 1.183, $P < 0.05$) in the nephropathic compared to the control group. The larger value for S reflects a broader distribution of pore size. As shown in Figure 3, the predominant change in nephropathic subjects is a relative increase in pores of smaller radius. However, a minor trend towards larger pores >70 Å radius can also be discerned at the upper end of the distribution (Fig. 3), and could have contributed to the elevated fractional clearances of albumin and IgG in the nephropathic subjects. In addition to the altered pore-size distribution, the model also reveals a loss of ultrafiltration capacity by glomeruli of nephropathic subjects. Median values for K_f in the latter were depressed below control values: 6.6 versus 9.8 ml/(mm · mm Hg), respectively ($P < 0.05$, Table 2). Corresponding values for S′/l were 208 versus 283 km, respectively (P = NS).

Longitudinal observations

As stated previously, 30 members of the original cohort of 34 subjects completed a re-evaluation of glomerular function after 48 months. They participated irregularly in the intervening six monthly studies, however, with the result that the group, as a

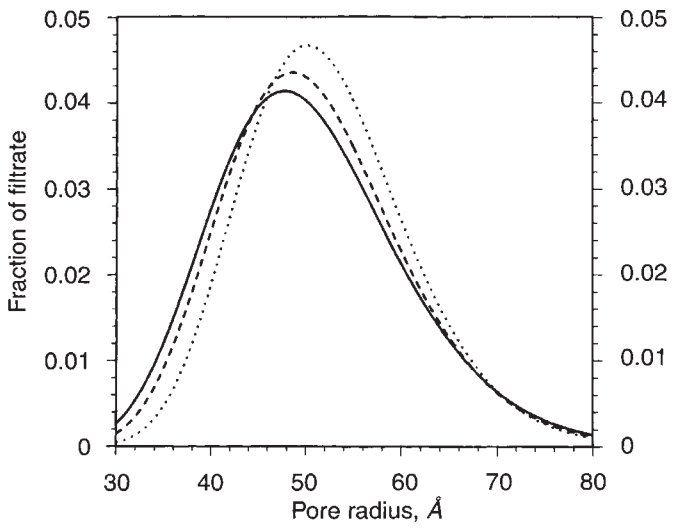

Fig. 3. *Lognormal distribution of pore radii in macroalbuminuric subjects at baseline (dashed) and after 48 months (solid) vs. normoalbuminuric controls (finely dotted).*

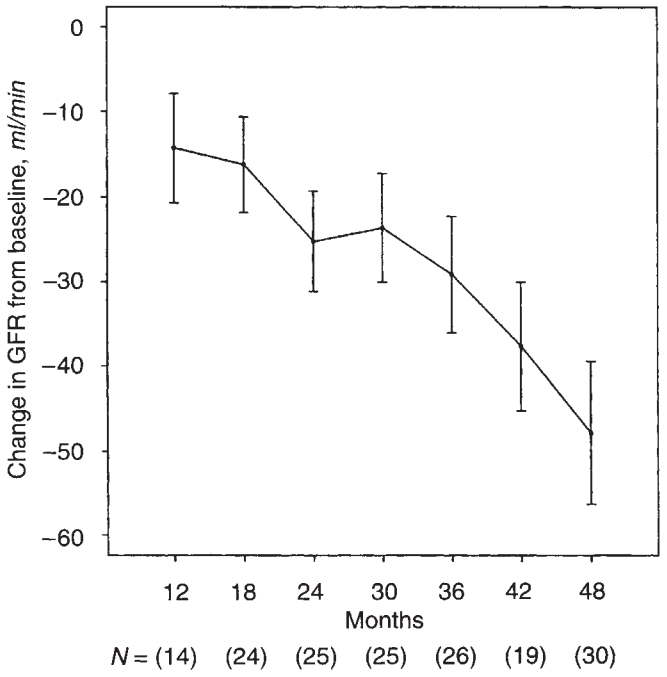

Fig. 4. *Mean (±SE) change in GFR from baseline in nephropathic group during 48 months of follow-up.* The number of observations at each interval is in parentheses below the x-axis. All changes between 12 and 48 months are significant ($P < 0.05$).

whole, underwent an average of six serial clearance studies (range 3 to 9). Two approaches which take missing data into account were used to estimate the course of GFR over the period of observation (see *Statistical methods*). The mixed effect model revealed a significant decline in GFR below baseline at all time points from 12 months onwards ($P < 0.05$) and a trend towards a progressively greater decline in GFR with the passage of time (Fig. 4). As described in the **Methods** section, ACEi therapy was initiated in 13 subjects during the course of our observations, and eight other subjects received therapy with β blockers and/or diuretics. As a result, serial determinations of blood pressure did not differ significantly from baseline throughout the 48 months of follow-up (Fig. 5). Nevertheless, the average decline below baseline for GFR at the end of follow-up was by 42 ml/min.

The course of GFR was also evaluated by using linear regression analysis to derive a GFR slope from the serial determinations [31]. Linear regression was also used to derive a slope for the natural log of the corresponding fractional protein clearances. As shown in Figure 6, most subjects exhibited a negative slope for GFR, and a positive slope for fractional protein clearance. The inverse relationship was highly significant for the slope of GFR versus that of both fractional albumin clearance ($r = -0.69$, $P < 0.001$, not shown) and fractional IgG clearance ($r = -0.74$, $P < 0.001$, Fig. 6). Thus, increasing damage to glomerular capillary walls in these nephropathic subjects was characterized by a trend towards declining GFR and increasing permeability to albumin and IgG.

Findings in the 30 subjects completing the 48 month study were compared to corresponding findings at baseline. Neither weight, BMI nor fasting glucose at the 48 month examination differed significantly from baseline. On average, however, glomerular dysfunction was substantially more marked at the 48 month than at the baseline examination. The A/Cr and IgG/Cr ratios increased by ~threefold, and the corresponding fractional clearances of each protein were enhanced by an order of magnitude (Table 3). Mean GFR declined significantly by 35% from 108 ± 6

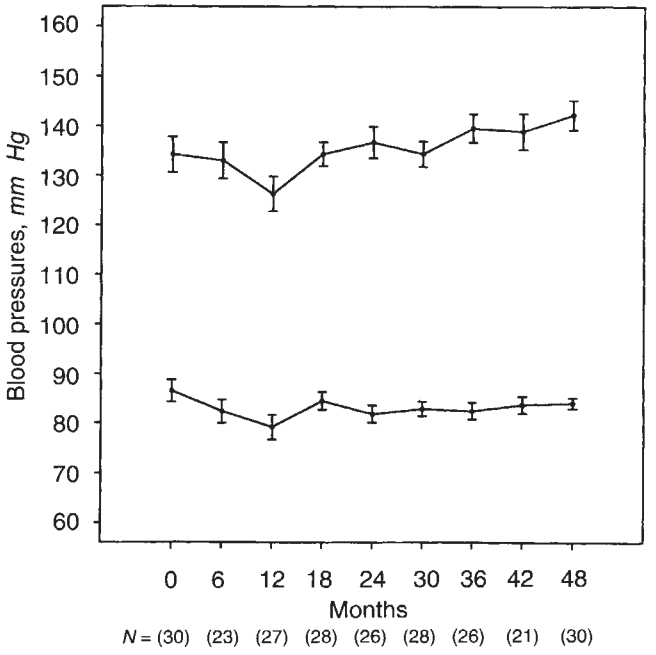

Fig. 5. *Serial determinations of systolic and diastolic blood pressures in the nephropathic group.* The number of observations at each interval is in parentheses below the x-axis. None of the serial determinations differ significantly from baseline.

to 71 ± 9 ml/min/1.73 m^2. Mean RPF also declined significantly, but less than in proportion to the GFR. As a result the filtration fraction was significantly lower at the 48 month examination than

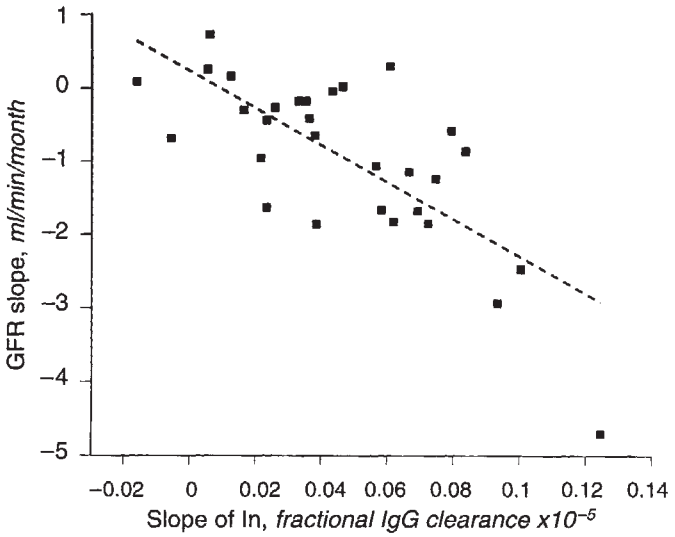

Fig. 6. *Relationship between the GFR slope (ml/min/month) and the corresponding slope of the natural log of fractional IgG clearance in the nephropathic group.* The dashed line is the regression line ($r = -0.74$, $P < 0.001$).

Table 3. Glomerular function in nephropathic subjects at baseline and after 48 months

	Baseline	48 Months
Albumin-to-creatinine ratio *mg/g*[a]	1036 (333–8944)	2824 (170–13,000)[b]
IgG-to-creatinine ratio *mg/g*[a]	131 (25–1332)	434 (26–3567)[b]
Fractional clearance of albumin $\times 10^{-3}$[a]	0.148 (0.030–6.73)	1.51 (0.032–56.6)[b]
Fractional clearance of IgG $\times 10^{-3}$[a]	0.050 (0.012–2.68)	0.635 (0.016–32.7)[b]
GFR *ml/min/1.73 m²*	108 ± 6	71 ± 9[b]
RPF *ml/min/1.73 m²*	666 ± 30	495 ± 43[b]
Filtration fraction	0.16 ± 0.006	0.14 ± 0.007[b]
π_A *mm Hg*	21.1 ± 0.4	20.5 ± 0.6
MAP *mm Hg*	102 ± 2	103 ± 2
Estimated K_f *ml/min · mm Hg*[a]	7.1 (6.0–8.9)	3.8 (2.3–6.7)[b]

[a] Median (range)
[b] $P < 0.05$ vs. baseline value

at baseline (Table 3). Neither π_A nor MAP changed significantly between the two examinations.

The increase in the urinary excretion and fractional clearance of protein after 48 months was accompanied by an alteration in the dextran sieving profile. A non-significant trend towards restricted transglomerular passage was evident for dextran molecules of < 40 Å radius. In contrast, the passage of larger molecules was enhanced, and the sieving coefficients for dextrans of 58 and 60 Å radius were significantly elevated above corresponding baseline values at the 48 month examination (Fig. 7). We used the observed values for dextran sieving coefficients, GFR, RPF, and π_A and assumed ΔP = 40 mm Hg to compute the prevailing membrane parameters. The median pore density declined significantly from 212 at baseline to 122 km at the 48 month examination ($P < 0.05$). A parallel decline by ~50% was computed for median K_f (Table 3). There was also a further alteration in

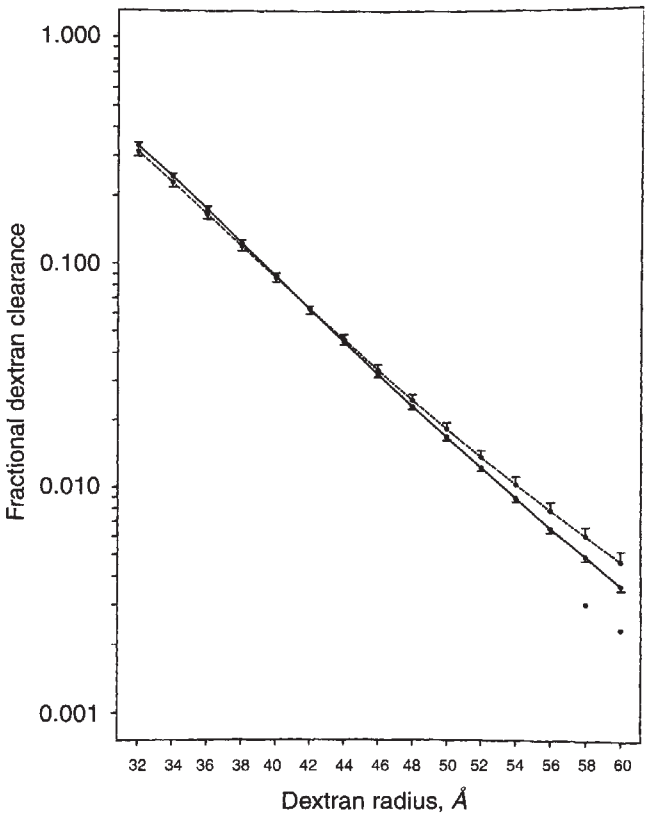

Fig. 7. *Dextran sieving curves in macroalbuminuric subjects at baseline (solid) and after 48 months (dashed) (*P < 0.05 vs. baseline).*

pore-size distribution which is illustrated in Figure 3 to permit comparison to baseline. The peak radius (u) was shifted to yet a smaller size (42.4 vs. 43.8 Å), and the distribution of pores (S) was broadened (S = 1.219 vs. 1.204, $P < 0.05$). The broadened pore-size distribution at 48 months included pores of larger as well as smaller radius than were evident at baseline. Of note, the fraction of filtrate that permeated pores > 70 Å radius at 48 months in the nephrotic subjects was twofold larger than the corresponding fraction in normoalbuminuric controls (Fig. 3). Thus, according to this analysis, the declining GFR in these nephropathic patients can be attributed to a progressive loss of intrinsic ultrafiltration capacity. A simultaneous broadening of glomerular pore-size distribution to include a greater fraction of pores of above 70 Å radius could have contributed to the increasingly heavy proteinuria observed over time (Fig. 3).

Discussion

We have recently reported that hyperfiltration is evident soon after the onset of NIDDM in the Pima Indians [12]. The finding in the present study that long-standing NIDDM in the normoalbuminuric subjects of the control group continues to be associated with an elevated GFR is of considerable interest. It is reminiscent of IDDM [1] and suggests that hyperfiltration is a non-specific characteristic of the diabetic state, and one that is independent of the etiology of the diabetes [3]. Our finding that the baseline GFR

in the nephropathic group was not similarly elevated suggests that the development of macroalbuminuria coincided with an alteration in one or more of the determinants of GFR.

Neither changes in RPF nor in π_A can be invoked to explain an initial value for GFR that was lower in nephropathic subjects than controls (Table 2). To the contrary, a trend to lower π_A and a significant reduction of the filtration fraction must have lowered the intraluminal glomerular capillary oncotic pressure in the nephropathic group relative to control values [33]. Further, the finding that arterial pressure was 12 mm Hg higher in the nephropathic than in the control group makes it unlikely that P could have been depressed [29, 30]. By exclusion, we infer that the lower GFR and filtration fraction observed in the nephropathic subjects is most likely to reflect a decline in the glomerular ultrafiltration coefficient, K_f [33].

That K_f was indeed lower in the nephropathic subjects is supported by the finding that the passage of dextrans of < 50 Å radius was restricted [26]. Application of a hydrodynamic theory of solute transport through a heteroporous membrane attributed this phenomenon to two alterations in intrinsic membrane properties. One was a reduction of mean pore radius. The other was a 33% depression of K_f (Table 2). A third membrane parameter, S'/l, was derived from K_f and pore radius [26]. It represents the ratio of effective area-to-pore length and was numerically reduced by 27%. Provided that pore length did not differ between control and nephropathic subjects, this implies that the total number of glomerular pores was lower in the latter group. Thus, whether expressed as an ultrafiltration coefficient or as pore density, the restricted dextran sieving in nephropathic patients points to a loss of intrinsic ultrafiltration capacity by glomerular capillary walls.

The K_f in the present study has been defined as the product of hydraulic permeability and the total surface area available for filtration in all glomeruli in the kidneys of each subject. Morphometric analyses of glomeruli obtained by biopsy from patients with early nephropathy complicating IDDM have revealed a reduction in both of the foregoing terms. Global sclerosis of a substantial fraction of glomeruli in such biopsies has attested to one obvious mechanism by which filtration surface is curtailed [34–36]. Another reported finding that would further limit filtration surface area is an impingement on the peripheral glomerular capillaries of patent glomeruli by an expanding mesangium [34–37]. The glomerular basement membrane and the interpodocytic slit diaphragms have been shown to be the major determinants of hydraulic permeability, with each structure accounting for approximately 50% of resistance to transcapillary water flow [38]. Morphometric studies have shown the basement membrane to be invariably thickened in early diabetic nephropathy [34–37]. Similarly epithelial foot processes have been shown to be broadened, leading to a reduction in the frequency of filtration slits and by extension, of the associated slit diaphragms [34, 39]. Together these latter two alterations are predicted to lengthen the filtration pathway, and hence to lower hydraulic permeability. Preliminary study of glomerular morphometry in the nephropathic subjects of the present study has revealed the same constellation of structural alterations as reported in IDDM [40]. Thus, it seems likely that a simultaneous reduction in both filtration surface area and hydraulic permeability provide the basis for the depressed K_f that we compute to have prevailed at the onset of the nephropathy in our patients with NIDDM. Given constancy of arterial pressure and a trend to lower π_A, we infer that increasing derangement of glomerular structure and ultrafiltration capacity is likely also to have accounted for the progressive decline in the GFR and filtration fraction that we observed during the 48 months of follow-up (Table 3).

Our theoretical analysis of the nephropathic dextran sieving profile suggests a modest impairment of barrier size-selectivity [41]. This is attested to by the sieving behavior of glomeruli towards the largest dextran molecules. Whereas the sieving coefficients for smaller dextrans were depressed relative to values in normoalbuminuric control subjects with NIDDM, the glomerular capillary walls of nephropathic subjects at baseline exhibited a selective failure to restrict the passage of large, nearly impermeant dextrans of > 50 Å radius (Fig. 2). This was followed after 48 months by a selective enhancement of passage of the largest dextran molecules (Fig. 7). This serial change in dextran sieving is interpreted to reflect a progressive broadening of pore-size distribution in nephropathic subjects. Whereas the shift in pore-size distribution was mostly towards pores of smaller radius, there was also a trend towards increasing prominence of large pores of > 70 Å radius (Fig. 3). Because of a configurational difference from proteins, dextran sieving coefficients overestimate the radius of pores encountered by proteins as they cross the glomerular barrier [42, 43]. Nevertheless, pores that present a radius of > 70 Å radius to permeating dextrans are likely to be permeable also to albumin (radius = 36 Å) and IgG (radius = 55 Å), and could contribute to the enhanced transglomerular passage of each protein that was observed. However, the increment in large pores that are potentially protein-permeable appears to be too small to account for the large disparity in protein clearance between the nephropathic and control subjects of the present study.

In addition to restricting circulating proteins on the basis of their size, the glomerular capillary wall also behaves as a negatively-charged electrostatic barrier [44, 45]. Studies in animals and humans with diabetic nephropathy have demonstrated a depletion of the fixed, negatively-charged sites within the glomerular capillary wall that normally impose this charge-selectivity [44–48]. It is therefore conceivable that a similar depletion in our nephropathic subjects contributed to the observed level of albuminuria. An isolated loss of barrier charge-selectivity cannot be invoked to explain the observed level of immunoglobulinuria, however. Not only are IgG molecules considerably larger than albumin molecules, but they also bear a predominantly positive charge [23]. Thus, impairment of size- rather than charge-selectivity appears to be required to explain the heavy and increasing immunoglobulinuria in the nephropathic subjects of the present study. To the extent that impaired charge-selectivity might have contributed to the observed level of albuminuria, the strong relationship between the fractional clearances of albumin and IgG in the nephropathic subjects (Fig. 1) suggests that the charge- and size-selective properties of the glomerular barrier must have deteriorated in parallel. This relationship is strongly reminiscent of the alteration in barrier function observed in the nephropathy of IDDM, and suggests a common mechanism of proteinuria in each form of diabetic nephropathy [34, 41, 49].

A final similarity between the nephropathy of these subjects with NIDDM and that reported for patients with IDDM is a downhill course characterized by a progressive impairment of ultrafiltration capacity and barrier function over time [34, 50]. On the other hand, an intriguing difference is our observation in the nephropathy of NIDDM of a trend for GFR to decline more

rapidly than is usually reported for the nephropathy of IDDM. Despite the maintenance of arterial pressure within a nearly normal range throughout the study (Fig. 6) and the administration of a converting enzyme inhibitor to a subset of the patients, the GFR declined by 37 ml/min over 48 months, on average. The approximate annual decline rate of 9 ml/min/year is two to three times more rapid than has been reported under similar conditions for the nephropathy of IDDM [51, 52]. This raises the possibility that Pima Indians with NIDDM might be susceptible to other factors that accelerate the progression of nephropathy. Less efficient control of hyperglycemia could be one such factor [53]. Another is the finding that Pima Indians, whether diabetic or non-diabetic, have glomeruli which are at least twofold larger than in Caucasian populations [54]. Further study will be required to confirm that the downhill course of nephropathy in Pima Indians with NIDDM is indeed more rapid than that seen in IDDM, and to elucidate the possible factors that might contribute to such rapid progression.

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